Peer Review File

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Responds to Reviewer A:

The manuscript is well written and provides novel data to prognosticate BCR after RP for prostate cancer treatment. The enrichment analysis is informative, adding strength to the study findings.

Minor Comments:

Comment 1. Line 205: cohort "was" stratified.

Reply 1: Thank you very much for your comment. We have replaced the “were” with “was”. In addition, we have sent the revised manuscript to the language editing company to improve the article for language and style.

Changes in the text: (line 234)

Comment 2. The Genomic Health OncotypeDx Prostate Cancer Assay is not mentioned in Background as another tool that has been validated to predict BCR after RP and should be added.

Reply 2: Thanks for the reviewer’s excellent comment. We added the Genomic Health OncotypeDx Prostate Cancer Assay as a tool to predict BCR after RP (Knezevic et al., 2013).

Changes in the text: (line 94)

Comment 3. Line 329: "In few words" could be removed.

Reply 3: Thanks for the excellent advices. We have removed "In few words".

Changes in the text: (line 367)

Comment 4. In Table 1, the racial composition of the cohort should be provided.

Reply 4: Thanks for the kind suggestion. We have added the racial composition of the cohort (Table 1). As shown in revised Table 1, BCR of PCa was associated with racial difference according to the result of Chi-square test ($P<0.0001$).

Responds to Reviewer B:

Comment 1. Grammatical editing is required throughout the paper.

Reply 1: Thank you very much for your comment. We have sent the revised manuscript to the language editing company to improve the article for language and style.

Comment 2. Abstract – the number of patients included from each dataset should be stated in the results.

Reply 2: Thanks for the insightful advice. We have added the number of patients included from each dataset in abstract.

Changes in the text: (lines 54-56)

Comment 3. In the first section of results (page 6, lines 183-185), this should start with a discussion of the findings with reference to the figures and tables rather than a paragraph simply stating what can be found in each table/figure.

Reply 3: Thank you very much for your comment. Considering the reviewer's suggestion, we have described the findings with reference to the figures and tables.

Changes in the text: (lines 194-207)

Comment 4. Figure 1 - explanatory text for this "study flow diagram" is needed. It is unclear which circles on the Venn diagram refer to which dataset and this needs to be clarified.

Reply 4: Thanks for the kind suggestion. We have added the explanation for the flow diagram. The blue, yellow, green and red circles refer to the TCGA, GSE21034, GSE70770 and GSE116918 datasets, respectively, and we have shown that in the revised Figure 1.

Changes in the text: (lines 194-200) (lines 478-480)

Comment 5. Figure 2/Results (lines 195-198) - an explanation of the LASSO analysis for a clinical audience is required here so as to more clearly understand the significance of the mRNAs identified.

Reply 5: Thanks for the reviewer's excellent comment. We have added an explanation of the LASSO analysis. The LASSO analysis was used to shrink all regression coefficients towards zero and to select variables simultaneously; and the minimum criteria as the optimal lambda values were determined through 10-fold cross-validations.

Changes in the text: (lines 220-223)

Comment 6. While the risk score was compared by ROC analysis to the individual clinical prognostic factors (PSA, T stage, GS) the paper would be strengthened by a comparison of the mRNA risk score/ nomogram with other established nomograms (e.g. CAPRA) rather than single factors alone. Ideally this would be a comparison by C-index.

Reply 6: Thanks for the reviewer's excellent comment. We use the "survcomp" package to perform the comparison of C-index between the 9-mRNA signature/nomogram and D'Amico model (D'Amico et al., 1998) which was developed based on clinical T stage, preoperative PSA, Gleason score. The University of California, San Francisco-Cancer of the Prostate Risk Assessment (UCSF-CAPRA) score was developed based on T stage, preoperative PSA, Gleason score, biopsy results and age (Cooperberg et al., 2005), but the TCGA and GSE21034, GSE70770 and GSE116918 datasets have no information of biopsy results. Therefore, we perform the comparison of C-index between the 9-mRNA signature/nomogram and D'Amico model.

Changes in the text: (lines 150-152, lines 244-246, lines 266-270, lines 281-283, Table S4 and Table S5)

Comment 7. P7 line 219 – the reference to K-M curves showing prognosis according to risk score should refer to figure 3 not figure S2 (this has K-M curves according to individual clinical risk factors)

Reply 7: Thanks for the kind suggestion. We have revised in manuscript.

Changes in the text: (lines 249-251)

Comment 8. P8 lines 261-262 – this sentence does not make grammatical sense.

Reply 8: Thanks for the kind suggestion. We have revised in manuscript.

Changes in the text: (lines 299-301)

Comment 9. In the discussion (p10, lines 299-302) the direct comparison of the AUC of this signature versus a previously published signature is not valid as they were performed on different cohorts of patients. In particular, it's performance in relation to the TCGA cohort will be biased given it was developed from that cohort.

Reply 9: Thanks for the kind suggestion. We have revised this part in the discussion.

Changes in the text: (lines 342-344)

Comment 10. In Discussion (p10, lines 304-6) the suggestion that the signature could alter treatment is also not valid with this level of evidence. The data presented here has provided some evidence of clinical validity (i.e. separates out 2 prognostic groups) but does not provide any evidence of clinical

utility (i.e. that changing treatment in either group improves patient outcomes). Instead a discussion of how this signature could be tested further in clinical trials would be more useful here.

Reply 10: Thank you very much for the reviewer's insightful comment. We have revised this part in the discussion. The 9-mRNA signature needed more clinical trials to validated its reliability in the future and we will conduct a long-term follow-up of PCa patients to collect their postoperative prognosis and survival time, and further verify our nomogram.

Changes in the text: (lines 344-346)

Reference:

Knezevic, D., Goddard, A.D., Natraj, N., Cherbavaz, D.B., Clark-Langone, K.M., Snable, J., et al. (2013). Analytical validation of the Oncotype DX prostate cancer assay - a clinical RT-PCR assay optimized for prostate needle biopsies. *BMC genomics* 14**,** 690. doi: 10.1186/1471-2164-14-690.

D'Amico, A.V., Whittington, R., Malkowicz, S.B., Schultz, D., Blank, K., Broderick, G.A., et al. (1998). Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 280(11)**,** 969-974.

Cooperberg, M.R., Pasta, D.J., Elkin, E.P., Litwin, M.S., Latini, D.M., Du Chane, J., et al. (2005). The University of California, San Francisco Cancer of the Prostate Risk Assessment score: a straightforward and reliable preoperative predictor of disease recurrence after radical prostatectomy. *The Journal of urology* 173(6)**,** 1938-1942.